REMARKS

I. Status Summary

Claims 1-8 are pending in the present U.S. patent application and have been examined by the United States Patent and Trademark Office (hereinafter "the Patent Office").

Claims 1-8 have been rejected under 35 U.S.C. §103(a) upon the contention that the claims are unpatentable over Ivanova and Belyavsky (1995) 23 *Nucleic Acids Research* 2954-2958 (hereinafter "Ivanova") in view of Kato (1995) 23 *Nucleic Acids Research* 3685-3690 (hereinafter "Kato").

Claims 1-8 also have been rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-43 of U.S. Patent No. 6,727,068 (hereinafter "the '068 Patent") in view of Ivanova.

Claim 1 has been amended. Support for the amendment can be found throughout the specification as filed, including particularly at page 6, line 28 through page 7, line 6; at page 8, lines 6-9; and at page 9, lines 1-5. Additional support can be found in the Figures, particularly Figure 3, which depicts quantitation of individual amplified fragments. Thus, no new matter has been added as a result of the amendments to claim 1.

A Terminal Disclaimer over the '068 Patent is being submitted herewith.

Reconsideration of the application in view of remarks set forth herein below in conjunction with the Amendments and Terminal Disclaimer filed herewith is respectfully requested.

II. Rejection under 35 U.S.C. § 103(a) over Ivanova *in view of* Kato

Claims 1-8 have been rejected under 35 U.S.C. §103(a) upon the contention that the claims are obvious over Ivanova in view of Kato. According to the Patent Office, Ivanova teach a method of claim 1 for sequence-specific identification, separation, and quantitation of an amplified subset of restriction fragments in a population of restriction fragments. The Patent Office concedes that Ivanova does not teach the use of restriction endonucleases having degenerate recognition or cleavage sequences, but asserts that this deficiency is

cured by Kato, which is asserted to teach a method for characterizing an mRNA population using class IIS restriction fragments.

The Patent Office further alleges that it would have been *prima facie* obvious to one of ordinary skill in the art to modify the method of Ivanova with a step including the use of type IIS restriction enzymes as taught by Kato in order to enhance the specificity of detecting a subset of an RNA population based on differential gene expression. The Patent Office further contends that one of ordinary skill in the art would have been motivated to combine the teachings of Ivanova with the method of Kato to arrive at the presently claimed subject matter because Kato allegedly explicitly teaches that the use of type IIS restriction enzymes for generating restriction fragments would discriminate and display each expressed gene in said RNA population, would reduce redundancy, and would provide an ideal method to generate tags for expressed genes that are used in characterizing an mRNA population. See Final Official Action at pages 3-6.

After careful consideration of the rejections and the Patent Office's basis therefor, applicants respectfully traverse the rejections and submit the following remarks.

Initially, applicants respectfully submit that the instant rejection appears to be based on the Patent Office's assertion that Ivanova taught the method of claim 1 save for the use of a restriction enzyme with a degenerate recognition or cleavage sequence, and that Kato teaches using such an enzyme. However, applicants respectfully submit that there are additional differences between the claimed subject matter and the cited combination of Ivanova and Kato that demonstrate that the claimed subject matter as a whole would not be obvious over the combination of Ivanova and Kato.

To elaborate, applicants respectfully submit that Ivanova at best discloses methods for identifying differentially expressed genes by fingerprinting the 3' ends of cDNA molecules. The technique is based on the isolation of 3'-end fragments that correspond to a fragment that extends from the poly-A tail to the first restriction site for a given enzyme that is 5' to the poly-A tail for each cDNA synthesized. This is demonstrated particularly in Figure 1 of Ivanova, which shows the reverse transcription of mRNAs using a biolinylated

oligo (dT) primer, the digestion of the resultant cDNAs with a "primary" restriction enzyme, and the purification of the 3'-end fragments with streptavidin microbeads.

Thus, for each and every cDNA generated, a 3'-end fragment is immobilized on the streptavidin beads. To these immobilized fragments an adapter is then added, but applicants respectfully submit that the adapter that is added will bind to each and every immobilized fragment. Thus, the adaptors employed in Ivanova do not fractionate the cDNA fragments as they do in the instantly claimed methods. Rather, in Ivanova any fractionating is accomplished by digesting the immobilized fragments sequentially with one or more additional restriction enzymes, with the resulting fragments of each such successive digestion being run on a polyacrylamide gel to create a fingerprint.

This is unlike the strategy recited in the instantly claimed methods. First, the instantly claimed methods do not rely on restriction digestion to fractionate the cDNAs. Rather, steps (c) and (d) of the method of claim 1 result in a direct fractionation of the cDNAs by ligating different adaptors to different subsets (*i.e.*, fractions) of digestion products, and amplifying only those fragments that have been ligated to adaptors. Thus, applicants respectfully submit that the instantly claimed methods employ adaptor ligation and amplification to fractionate the fragments and not restriction digests *per se*.

Additionally, applicants respectfully submit that the Patent Office has misinterpreted the disclosure of Ivanova in its assertion that Ivanova discloses (c) ligating said restriction fragments to adaptors lacing restriction endonuclease sites, wherein each adaptor has a sequence complementary to one of said overhangs, wherein each ligating reaction is performed with one adaptor that can be ligated to a subset of said restriction fragments (emphasis added). Applicants respectfully submit that it is clear from the Ivanova reference that the adaptors that are used are ligated to each and every immobilized fragment since only one restriction enzyme is employed and there is no disclosure in Ivanova that this restriction enzyme can be one that employs a degenerate recognition or cleavage sequence.

Thus, Ivanova discloses a method wherein one type of adaptor is ligated to each and every member of a plurality of immobilized fragments, and further that the adaptor is ligated

to only one end (*i.e.*, the end that corresponds to the 5'-end of the mRNA) of each member of the plurality of immobilized fragments. Accordingly, the adaptor is not ligated to a subset of the produced restriction fragments.

Furthermore, applicants respectfully submit that claim 1 has been amended to clarify that each adapter has a sequence complementary to one of said N^m different single-stranded overhangs. Applicants respectfully submit that since claim 1 recites that N is 2-4 and m is 1-5, the claim explicitly recites that at least two different overhangs are produced. Applicants respectfully submit that Ivanova does not teach this aspect of claim 1.

In addition, the Patent Office appears to assert on page 4 of the Final Official Action that Ivanova teach that the method comprises further digesting the restriction fragments with one or more additional restriction enzymes and ligating adaptors. Applicants respectfully submit that Ivanova does not disclose digesting the restriction fragments with one or more additional restriction enzymes and then ligating adaptors as recited in claims 4 and 5. Applicants respectfully submit that claims 4 and 5 include a temporal element in that claim 4 requires the product of step (b) of claim 1, and claim 5 requires the product of step 4.

Thus, claim 4 recites obtaining the restriction fragments produced by digesting the cDNA molecules with one or more restriction endonucleases having a degenerate recognition or cleavage sequence as per claim 1 step (b), and digesting these restriction fragments with one or more further restriction endonucleases to produce a plurality of restriction fragments with one or more single-stranded overhangs that are different from those produced in (b). Claim 5 then recites ligating the single-stranded overhangs produced by the digesting of claim 4 to a series of adapters, each adapter having a sequence complementary to one of said overhangs.

As such, applicants respectfully submit that claims 4 and 5 recite methods in which certain steps occur in the following order: (i) digesting cDNA molecules with one or more restriction endonucleases having a degenerate recognition or cleavage sequence; (ii) digesting these restriction fragments with one or more further restriction endonucleases to produce a plurality of restriction fragments with one or more single-stranded overhangs that

are different from those produced in the previous step (this is claim 4); and (iii) ligating the single-stranded overhangs produced by the digesting of step (ii) to a series of adapters, each adapter having a sequence complementary to one of said overhangs. Applicants respectfully submit that Ivanova does not disclose or suggest this series of ordered steps.

In fact, applicants respectfully submit that the method disclosed in Ivanova would not be operable if this series of ordered steps were employed. Particularly, applicants respectfully submit that the method of Ivanova that requires that the overhangs produced all be identical so that the amplification reaction can employ the adaptor sequence and the oligo (dT) sequence as primer sequences. If multiple enzymes were employed before the ligation reaction, the fragments that included the oligo (dT) sequence would be expected to have different 5' ends. As a result, the adaptors would not bind to all of the fragments produced and the amplification reaction would not amplify each and every fragment that included the oligo (dT) sequence. Therefore, applicants respectfully submit that the Patent Office's apparent assertion that Ivanvoa teaches the method of claims 4 and 5 is believed to be contrary to the disclosure of Ivanova and thus inaccurate.

And finally, applicants respectfully submit that the Patent Office has misinterpreted Figure 4 of Ivanova in asserting that this Figure discloses a quantifying step. Applicants respectfully submit that Figure 4 of Ivanova relates to an assessment of the distribution of fragments of various lengths when different restriction enzymes or combinations of restriction enzymes are employed. Applicants respectfully submit that this length distribution is not equivalent to the detecting and quantifying step of claim 1, which relates to quantitating an amount of a fragment that results from the amplification step of claim 1. *See also* page 6, line 28, through page 7, line 6, of the specification as filed (including particularly, page 7, line 6). As such, applicants respectfully submit that Ivanova does not provide a quantifying step as recited in claim 1.

Therefore, applicants respectfully submit that it is clear that the methods disclosed in Ivanova and recited in the instant claims differ in many respects other than just the use of a restriction enzyme having a degenerate recognition or cleavage sequence. Applicants further respectfully submit that these differences are not cured by the Kato reference.

Particularly, applicants respectfully submit that even if the method of Ivanova was modified to include the use of restriction enzymes having a degenerate recognition or cleavage sequences as suggested by the Patent Office, the method would still be limited to amplification of restriction fragments at the 3'-end of cDNAs based on the fact that both Ivanova and Kato teach methods that are limited to analysis of 3'-end fragments of cDNAs.

In contrast, as set forth in step (d) of instant claim 1, the amplifying step comprises amplifying a subset of said restriction fragments for no more than 25 cycles with a primer comprising a detectable label, wherein said primer is designed to amplify only those restriction fragments to which said one adapter of said series of adapters has been ligated. Stated another way, the amplification step employs a single primer sequence that bind to a sequence present within the adaptor. To successfully amplify a fragment using a single primer sequence, the single primer sequence must be present at each end of the fragment.

Therefore, the amplification step recited in step (d) relates to an amplification of a subset of the fragments generated by cutting the cDNA population with the degenerate restriction enzyme, wherein the subset of fragments is characterized by having the adapter sequence at each end.

Stated another way and by example, if the degenerate restriction enzyme has a two base overhang that includes complete degeneracy over the two bases (an example of this is *BsaJ*I), then there are sixteen possible overhangs that can be generated. For each cDNA, there would be one fragment that corresponds to the 5' end of the cDNA to the 5' most cleavage site and one fragment that corresponds to the 3' end of the cDNA to the 3' most cleavage site. There would also be between 0 or more fragments that correspond to fragments that had (in this example) *BsaJ*I sites at both ends. For any given fragment, however, the probability that the overhangs at each end are the same are only 1 in 16, and the probability that the overhangs are both a particular member of the 16 possible overhangs is only 1 in 256 (16^2).

Thus, since each ligation step employs one class of adapter that can be ligated to one possible overhang, and the amplification step employs a primer that binds to the adapter sequence, the amplification step will amplify only those fragments that have

adapters at each end. Applicants respectfully submit that this is not taught in the combination of Ivanova and Kato, each of which employs an adapter at one end and a primer that binds to the poly-A tail of cDNA. Therefore, unlike the situation encountered in the instant method, the methods of Ivanova and Kato relate to using one primer that binds to every single fragment present in the amplification reaction, namely the oligo-dT primer.

Thus, applicants respectfully submit that the instant rejection appears to be based on a consideration of the subject matter of the claims and of the cited references that does not consider either as a whole, and thus is improper under M.P.E.P. § 2141.02. To elaborate, applicants respectfully submit that M.P.E.P. § 2141.02 states that

> In determining the differences between the prior art and the claims, the question under 35 U.S.C. 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983); *Schenck v. Nortron Corp.*, 713 F.2d 782, 218 USPQ 698 (Fed. Cir. 1983)

M.P.E.P. § 2141.02 (emphasis added). Applicants respectfully submit that since neither Ivanova nor Kato teach or suggest amplifying a subset of fragments that have adapters ligated at both ends, the combination of Ivanova and Kato does not support a rejection under 35 U.S.C. § 103(a).

Accordingly, applicants respectfully submit that the combination of Ivanova and Kato does not support the instant rejection of claim 1. Applicants further respectfully submit that claims 2-8 all depend from claim 1, and thus are also believed to be distinguished over the cited combination. As a result, applicants respectfully request that the instant rejection of claims 1-8 be withdrawn at this time.

III. Obviousness-Type Double Patenting Rejection

Claims 1-8 also have been rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-43 of U.S. Patent No. 6,727,068 (hereinafter "the '068 Patent") in view of Ivanova. According to the Patent Office, it would have been obvious to one of ordinary skill in the art as of the filing date to combine the method taught in claims 1-43 of the '068 Patent with the step of including amplification for

no more than 25 cycles as taught by Ivanova for the purpose of enriching the amplification products exponentially. The Patent Office further asserts that one of ordinary skill in the art would have been motivated to combine the references because Ivanova explicitly taught that the exponential low-stringency amplification PCR would amplify products that enable one to identify and clone the fragments. *See* Final Official Action at pages 6-9.

In response to the Patent Office's assertions, applicants submit herewith a timely filed Terminal Disclaimer. Applicants respectfully submit that the Patent Office should not construe the enclosed Terminal Disclaimer as an acknowledgment or acquiescence in the accuracy of the instant rejection. Indeed, the Federal Circuit has noted that a Terminal Disclaimer "is not an admission of obviousness of the later filed claimed invention in light of the earlier filed disclosure for that is not the basis of the Disclaimer." Quad Environmental Technologies v. Union Sanitary District, 20 U.S.P.Q.2d 1392, 1394 (Fed. Cir. 1991).

The Federal Circuit further noted:

> In legal principle, the filing of a Terminal Disclaimer simply serves the statutory function of removing the rejection of double patenting and raises neither presumption nor estoppel on the merits of the rejection. It is improper to convert this simple expedient "obviation" into an admission or acquiescence or estoppel on the merit.

Quad Environmental Technologies, 20 U.S.P.Q.2d at 1394-95. Therefore, with the submission of the Terminal Disclaimer provided herewith, applicants are simply availing themselves of the statutory function of removing the double patenting rejection.

Accordingly, withdrawal of the obviousness-type double patenting rejection of claims 1-8 based on claims 1-43 of the '068 Patent is respectfully requested.

CONCLUSIONS

In light of the above amendments and the remarks presented hereinabove, it is respectfully submitted that claims 1-8 are in proper condition for allowance, and such action is earnestly solicited.

If any minor issues should remain outstanding after the Examiner has had an opportunity to study the Amendment and Remarks, it is respectfully requested that the

Examiner telephone the undersigned attorney so that all such matters may be resolved and the application placed in condition for allowance without the necessity for another Action and/or Amendment.

DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any deficiencies or credit any overpayments associated with the filing of this correspondence to Deposit Account Number **50-0426**.

Respectfully submitted,
JENKINS, WILSON, TAYLOR & HUNT, P.A.

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